REMARKS

Claim 1 has been amended to recite:

[a] process for the biological production of vitamin B_6 which comprises cultivating a host cell transformed or transfected by an isolated DNA or by a vector or plasmid comprising the isolated DNA under conditions conducive to the production of vitamin B_6, and recovering vitamin B_6 from the culture, wherein the host cell is selected from Sinorhizobium or Escherichia and wherein the isolated DNA comprises a nucleotide sequence encoding PdxR, which is a flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase, selected from the group consisting of:

(a) a DNA sequence identified by SEQ ID NO:1 or the complementary strand thereof;
(b) a DNA sequence which hybridizes under stringent hybridization and stringent washing conditions to the DNA sequence complementary to the DNA sequence defined in (a), and encodes a polypeptide having the activity of flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase, wherein the stringent hybridization conditions comprise hybridization in 2XSSC and 0.5% sodium dodecyl sulfate (SDS) at 45°C for 1 hour and wherein the stringent washing conditions comprise washing in 0.1X SSC and 0.5% SDS at 60°C for 1 hour;
(c) a DNA sequence encoding a polypeptide having the amino acid sequence encoded by the DNA sequence of (a), (b), or (d);
(d) a DNA sequence which is at least 95% identical to a DNA encoding a polypeptide which comprises the amino acid sequence of SEQ ID NO: 2, and encodes a polypeptide having the activity of flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase; and
(e) a DNA sequence encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO: 2, and encodes a polypeptide having the activity of flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase.

Support for these amendments may found in the original claim 1 and in the specification at, for example, page 3 lines 15-35; page 4 lines 1-8. *See In re Gardner*, 177 USPQ 396, 397 (CCPA 1973) and MPEP §§ 608.01 (o) and (l).

It is submitted that no new matter has been introduced by the foregoing amendments. Approval and entry of the amendments is respectfully solicited.

§112, SECOND PARAGRAPH REJECTIONS:

Claims 1-3 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite and vague in the recitation of "stringent hybridization and stringent washing conditions." (Paper No. 20070926 at 2). In making the rejection, the Examiner asserted that "claim 1 still reads on conditions that are not clearly defined." (*Id.* at 3). The Examiner further asserted that "although page 3 describe[s] a condition, the description is merely exemplary." (*Id.*).

As is well settled, all that is required to comply with 35 USC §112, second paragraph, is that the metes and bounds of what is claimed be determinable with a reasonable degree of precision and particularity. *Ex parte Wu*, 10 USPQ2d 2031, 2033 (BPAI 1989).

With a view towards furthering prosecution, part (b) of claim 1 has been amended, *inter alia*, to explicitly recite the intended stringent hybridization and stringent washing conditions. Hence, as amended, there is nothing vague or indefinite about any of the recited conditions in claim 1. One skilled in the art would readily recognize what is being claimed in claim 1, as amended.

In view of the foregoing, the indefiniteness rejections with respect to claims 1-3 have been rendered moot and should be withdrawn.

§112, FIRST PARAGRAPH REJECTION:

Claims 1-3 have been rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. (Paper No. 20070926 at 3).

In making the rejection, the Examiner acknowledged that "the specification ... [is] enabling for a process for the biological production of vitamin B6 comprising cultivating a Sinorhizobium cell transformed or transfected by a DNA molecule of SEQ ID NO: 1 from S. meliloti encoding a polypeptide of SEQ ID NO: 2 having D-erythronate-4-phosphate dehydrogenase activity." (*Id.* at 4)

The Examiner, however, asserted that the specification "does not reasonably provide enablement for a process for the biological production of vitamin B6 comprising cultivating a Sinorhizobium or Escherichia host cell transformed or transfected by any DNA molecule encoding any polypeptide, which is 80% identical to SEQ ID NO: 1 or SEQ ID NO: 2 having flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase activity." (*Id.*) The Examiner further asserted that "[t]he scope of the claimed invention is very broad in the context of at least 80% identity to SEQ ID NO: 1 or 2." (*Id.*) Additionally, the Examiner asserted that "[w]hile methods to produce variants of a known sequence such as site-specific mutagenesis, random mutagenesis, etc. are well known to the skilled artisan, producing variants useful as D-erythronate 4-phosphate dehydrogenase requires that one of ordinary skill in the art know or be provided with guidance for the selection of which of the infinite number of variants have the activity." (*Id.*)

The Examiner further asserted that Guo *et al.*, "Protein Tolerance to Random Amino Acid Change," *Proc Natl Acad Sci U S A*, 101 (25): 9205-10 (2004) ("Guo") discloses that "percentage of random single substitution mutations which

inactivate a protein for the protein 3-methyladenine DNA glycosylase is 34% and that this number appears to be consistent with other studies in other proteins as well." (Paper No. 20070926 at 5). The Examiner also asserted that, "Guo further showed in Table 1 that the percentage of active mutants for multiple mutants appears to be exponentially related to this by the simple formula $(.66)^x$ X 100% where x is the number of mutations introduced." (*Id.*) Applying Guo's formula, the Examiner asserted that "the instant protein 80% identity allows up to 99 mutations within the 496 amino acids of SEQ ID NO: 2 and thus only $(.66)^{99}$ X 100% or 1.3 x 10^{-16}% (i.e. ~ 1 in several billion) of random mutants having 80% identity would be active." (*Id.*) Although the Examiner acknowledged that "[c]urrent techniques (i.e., high throughput mutagenesis and screening techniques) in the art would allow for finding a few active mutants within several hundred thousand," the Examiner asserted that "finding a few mutants within many billions or more as in the claims to 80% or less identity would not be possible." (*Id.*) Moreover, the Examiner asserted that "if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has **not** been provided in the instant specification." (*Id.*, emphasis original) The Examiner, however, acknowledged that "enablement is not precluded by the necessity for routine screening." (*Id.*)

The Federal Circuit, adopting the analysis set forth in *Ex parte Forman*, 230 USPQ 546 (BPAI 1986), has enumerated several factors which may be considered in determining whether claims require that one skilled in the art perform undue experimentation in order to practice the claimed subject matter: breadth of the claims;

predictability or unpredictability of the art; relative skill of those in the art; state of the prior art; nature of the invention; working examples; amount of guidance; and quantity of experimentation necessary. *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). These factors are merely illustrative, not mandatory; they provide a general framework for analysis. *Enzo Biochem v. Calgene Inc.*, 188 F.3d 1362, 1371, 52 USPQ2d 1129, 1136 (Fed. Cir. 1999); *Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1213, 18 USPQ2d 1016, 1027 (Fed. Cir.), *cert. denied*, 502 U.S. 856 (1991).

In fact, enablement may still be present when an application contains no working examples or when prophetic examples are used. *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1576-77, 224 USPQ 409, 414 (Fed. Cir. 1984) ("Use of prophetic examples, however, does not automatically make a patent non-enabling.") and *Strahilevitz*, 668 F.2d at 1232, 212 USPQ at 563 ("Nevertheless, as acknowledged by the board, examples are not required to satisfy section 112, first paragraph.").

With a view towards furthering prosecution, parts (d) and (e) of claim 1 have been amended to recite:

> (d) a DNA sequence which is at least 95% identical to a DNA encoding a polypeptide which comprises the amino acid sequence of SEQ ID NO: 2, and encodes a polypeptide having the activity of flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase; and
>
> (e) a DNA sequence encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO: 2, and encodes a polypeptide having the activity of flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase.

Thus, as amended, claim 1 no longer reads on "any DNA sequence which is 80% identical to SEQ ID NO:1." (Paper No. 20070926 at 3).

As acknowledge by the Examiner, the disclosed D-erythronate 4-phosphate dehydrogenase is a polypeptide of 496 amino acids, and the DNA encoding the dehydrogenase is 1488 nucleotides (and with the stop codon, 1491 nucleotides). Thus, a claim of 95% identity to a DNA which is at least 95% identical to a DNA encoding a polypeptide which comprises the amino acid sequence of SEQ ID NO: 2 is not overly broad.

Furthermore, as the Examiner acknowledged, "methods to produce variants of a known sequence such as site-specific mutagenesis, random mutagenesis, etc. are well known to the skilled artisan" (Paper No. 20070926 at 4) and "[c]urrent techniques (i.e., high throughput mutagenesis and screening techniques) in the art would allow for finding a few active mutants within several hundred thousand" (*Id.* at 5). Thus, the Examiner appears to concede that the relative skill of a person skilled in the art is high, the art of mutagenesis and screening is fairly predictable, and the state of the prior art is reasonably advanced.

Additionally, the specification provides ample disclosure sufficient to make, screen and use a variant of flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase for the biological production of vitamin B_6. For example, the specification discloses four detailed examples and five tables that provide sufficient instruction for using such a variant. Specifically, the specification discloses, *inter alia*, how to isolate, clone and overexpress DNA of interest, assays to test the function of putative flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase, and host cells useful for the production of vitamin B6 for identifying enzymes which would fall under the currently claimed process. (*See, e.g.,* Specification at page 1, lines 15-21, page 2, lines 5-14, page 3, lines 18-19, page 4, lines 27-33,

page 6, lines 22 to 26, and in Examples 1-4). Given the disclosure, a person skilled in the art will be able to make the putative flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase (in accordance with procedures known in the art and the disclosed sequences), assay it for flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase function, and use it in the production of vitamin B_6.

Moreover, the specification of the instant application provides guidance as to what amino acid substitutions may be made without altering flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase function. For example, the last two sentences of the paragraph bridging pages 3 and 4 of the specification disclose, "[a]mino acid exchanges in proteins and peptides which do not generally alter the activity of such molecules are known in the state of the art. The most commonly occurring exchanges are: Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, Asp/Gly as well as these in reverse." (page 3, lines 33-34 and page 4, lines 1-3.) Thus, the specification provides specific guidance to appropriate amino acid substitutions.

Finally, we address the Examiner's arguments with respect to the Guo disclosure. Initially, we note that while the formula for the percentage of active mutants for multiple mutations cited by the examiner, $(0.66)^x$ X100%, wherein x is the number of mutations introduced, is applicable to human 3-methyladenine DNA glycosylase (AAG), the 0.66 value used in the formula varies significantly across various proteins, ranging from 0.44 to as high as 0.81. (*See e.g.*, Guo, page 9206, column 2, lines 27-30 and table 2). Furthermore, Guo discloses that "[t]here likely are variations in the

substitutability of different proteins." (Guo, page 9207, column 1, 32-33.) Hence, the formula cited by the Examiner is not likely to be applicable to the D-erythronate 4-phosphate dehydrogenase disclosed by the present application. And, indeed the Examiner has provided no evidence that such a formula would be relevant in the present context.

Furthermore, even assuming that the formula, $(0.66)^x$ X100%, accurately describes the percentage of active mutants for multiple mutations in the D-erythronate 4-phosphate dehydrogenase as disclosed by the Applicants, the amended claim 1 still would be enabling. The disclosed D-erythronate 4-phosphate dehydrogenase is a polypeptide of 496 amino acids, and 95% amino acid sequence identity means a mutation of only about 25 amino acids. Using the formula above, $(0.66)^{25}$ gives $3.08X10^{-5}$, or $3.08X10^{-3}$%. This number, which translates to 1 in 32,464, is well within the Examiner's acknowledged ability of the current art, which "allow[s] for finding a few active mutants within several hundred thousand." (Paper No. 20070926 at 5). Given that guidance is provided in the specification as to what substitution of amino acids may be made without altering the enzymatic activities, the probability of 1 in 32,464 should be further increased, and again, well within the ability of the current art. Therefore, as amended, claim 1 is enabling.

As acknowledged by the Examiner, "enablement is not precluded by the necessity for routine screening." (Paper No. 20070926 at 5). At bottom, the amount of experimentation required to achieve 95% identity with a DNA encoding a polypeptide which comprises the amino acid sequence of SEQ ID NO: 2, and encodes a polypeptide having the activity of flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase is not undue, particularly given that the Examiner appears

to concede that the techniques of mutation are well developed in the art, that the skill of artisans in this field is high, the capability of the current mutant screens are large, and that the specification includes assays and working examples as to how to assess the function of D-erythronate 4-phosphate dehydrogenase, as well as guidance to nucleotide changes.

For the reasons set forth above, it is respectfully submitted that the rejection has been overcome and should be withdrawn.

For the foregoing reasons, favorable action on the merits, including entry of the amendments, withdrawal of the objections and rejections, and allowance of all the claims, respectfully is requested. If the Examiner has any questions regarding this paper, please contact the undersigned attorney.

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop AF, Commissioner for Patents, P.O. Box. 1450 Alexandria, VA 22313-1450, on April 11, 2008.

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Respectfully submitted,

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